PHYTOCHEMICAL SCREENING AND TOXICITY OF ETHANOLIC EXTRACT OF MANGROVE (*Rhizophora mucronata*) LEAVES FROM LANGSA, ACEH TIMUR

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ABSTRACT

Mangrove has special properties, i.e. able to adapt to the extreme environment. Any kind of plant that able to adapt in such a condition usually can have a different metabolic pathway and as the impact, this plant can produce a different metabolite which can act as an antibiotic. The aim of this study was to evaluate the phytochemical content and toxicity properties of *Rhizophora mucronata*. The functional group that presence in the extract was determined using UV-Vis and FT-IR. The phytochemical screening showed the presence of alkaloid, flavonoid, quinone, tannin and phenolic. The UV-Vis spectrum also proved the presence of phenolic (tannin) and flavonoid in the *Rhizophora mucronata* extract (280-390 nm). The presence of hydroxyl, carboxylic acid, aromatic C=C group was confirmed from the FTIR spectrum. The toxicity property of ethanolic extract of *Rhizophora mucronata* showed LC₅₀ of 636.73 ppm $\leq$ 1000 mg/L.

Keywords: Mangrove, *Rhizophora mucronata*, Phytochemical, FTIR, UV-Vis, Toxicity

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INTRODUCTION

Mangrove is plant communities that grow in coastal saline or brackish water and functioned as protectors of the coastal area from erosion, storm surge and tsunami. Langsa is a city in Aceh province with 262.41 km² of area. The mangroves in-and-around the langsa city have decreased in the area from 9550 ha in 2013 to 7837 ha as of 2015, during this time commercial shrimp aquaculture has become the dominant land-cover within this estuary environment.¹

In Aceh, mangrove leaves have been utilized as a traditional medicine to cure several health issues, such as dysentery, bleeding, weight loss, antiseptic.

Mangrove plant has special ability to able to grow in an extreme environment, i.e. waterlogging, unstable and oxygen-deficient soils, and high salinity.² Any kind of plant that able to cope with this condition, usually will be able to have a new metabolic pathway and produce a specific metabolite that can be utilized to support its life.³ From the previous study, mangrove plants consisted of several secondary metabolites, such as alkaloid, saponin, tannin, and flavonoid. The presence of these metabolites can be used as an indication of the potency of mangrove as a medicine plant.⁴ Based on previous research, mangrove extract showed a potency as antimicrobe, antiviral, anticancer, and antidiabetic.⁵

The phytochemical screening and toxicity properties are common methods that can be used to discover new kinds of medicine. Toxicity is a degree to indicate which substance or chemical that can harm humans or animals. BSLT technique is a common method that can be used to identify the toxicity property of any chemical.

The objective of the current research was to identify the phytochemical content and the toxicity properties of ethanolic extract of *Rhizophora mucronata* from Langsa, Aceh Timur.

EXPERIMENTAL

Material and Method

Rhizophora mucronata leaves were collected from mangrove forest in Langsa, Aceh Timur. The obtained leaves were air-dried until the water content was $\pm 15\%$. The taxonomy of this plant was identified in Herbarium medanense (MEDA), Universitas Sumatera Utara, Indonesia.

General Procedure

The extraction process of *Rhizophora mucronata* was performed following the maceration technique of Baehaki Ace, 2017.⁶ The dried leaves were grounded to obtain powder formed. About 20 g of simplisia was macerated using 80 mL of 95% ethanol for 24 h while shaking in a shaker. The mixture was filtrated using Whatman No. 42. The obtained filtrate was then evaporated using a rotary evaporator until the concentrated extract was obtained. This extract was characterized for phytochemical screening, UV-Vis, FTIR and toxicity

Detection Method

Phytochemical Screening

The qualitative phytochemical screening of *Rhizophora mucronata* extract was performed using Harborne⁷ and Trease & Evans method.⁸

UV-Visible Spectrophotometer

The spectrum of a single eluted compound was further recorded between 280 to 450 nm on a UV-Visible Spectrophotometer (Thermo Scientific Inc., MA, and USA)

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the sample was recorded using a Bruker OPUS 7.5.18 infrared spectrometer (Bruker, Germany) at wavelengths from 400 to 4000 cm^{-1} at a speed of 20 $\text{cm}^{-1} \cdot \text{s}^{-1}$

BSLT

The toxicity of *Rhizophora mucronata* was evaluated using BSLT method. About 10 larvae of *A. salina* Leach that has been growing for 48 h was placed into a bottle that contained seawater and extract. The BSLT was performed triplicate for each group and observed for 24 h. The number of dead larvae was calculated, and the percentage of mortality was calculated. The obtained data were used to determine the LC_{50} .

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screening showed *Rhizophora mucronata* leaves that extracted using 96% ethanol consisted of several secondary metabolites, i.e. alkaloid, flavonoid, quinone, tannine, and phenolic. Phytochemical content in plants has a significant role in the antibacterial activity.⁹ Apart from that, plants that are composed of bioactive compounds such as flavonoids, alkaloids, saponins, phenolic vitamins, etc. also have biochemical activity.¹⁰ The screening result of *Rhizophora mucronata* leaves extract was shown in Table-1.

Table-1: Phytochemical Screening of *Rhizophora Mucronata* Crude Extract

Secondary metabolite	Result
Alkaloid	+
Steroid	-
Terpenoid	-
Flavonoid	+
Saponin	-
Quinone	+
Tannine	+
Qumarin	-
Phenolic	+

The presence of those metabolites can indicate a potency of *Rhizophora mucronata* as a new potential medicine. In the previous study, those metabolites can be used as an antibacterial agent that works in different ways. Alkaloid was identified can act as an interfere to disturb the formation of the cell wall and induced the dead cell. Flavonoid is a lypophilic compound that can easily interact with the phospholipid that can be found in the bacterial cell wall, as a result, the flavonoid can act as antibacterial.

Tannin has potency as a chelating agent with plasmolytic affect and disturbs the cell permeability. Tannin can as an antibacterial agent through protein precipitation, interact with the cell membrane, enzyme inactivation, destruction or inactivation of bacterial genetic material.¹¹ The antimutagenic activity of plants is due to the presence of total phenols and flavonoid content.¹²

UV-Visible Spectrophotometer

The UV-Vis spectrum of *Rhizophora mucronata* leaves extract is shown in Fig-1 that measured in the range 260-450 nm. The highest peak is observed at 288 nm with a value of 0.173. This result showed the presence of flavonoids in the extract.

Fig-1: UV-Vis Spectrum of *Rhizophora Mucronata* Leaves Extract

Flavonoid has a specific peak around 240-290 nm, this wide range is caused by the presence of conjugation bonding of ring structure.¹³⁻¹⁴

FTIR Analysis

The FTIR analysis was performed to determine the functional group that presence in the ethanolic extract of *Rhizophora mucronata* leaves. The FTIR spectrum of the *Rhizophora mucronata* is shown in Fig-2.

Fig-2: FTIR Spectrum of *Rhizophora Mucronata* Leaves Extract

The FTIR spectrum showed the presence of a typical peak of flavonoid, i.e. 3319, 2973, 1607, 1444, 1042, and 878 cm^{-1} . The band at 3319 is assigned to the presence of -OH stretching from phenolic. The presence of C-H alkenes is assigned by the band at 2973 cm^{-1} . While band at 1607 and 878 cm^{-1} are indicated to the presence of C=C from aromatic ring and C-O.¹⁵

BSLT (*Brine Shrimp Lethality Test*)

The toxicity of *Rhizophora mucronata* was determined using BSLT method. The mortality percentage and LC_{50} of ethanolic extract of *Rhizophora mucronata* are shown in Table-2.

Table-2: Mortality Percentage and LC_{50} of Ethanolic Extract of *Rhizophora Mucronata*

Concentration (ppm)	Dead	Larvae total	Mortality (%)	LC_{50} (ppm)
10	5	30	16,66	636,725
100	7	30	23,33	
1000	21	30	70,00	

Based on LC_{50} value, the ethanolic extract of *Rhizophora mucronata* is classified has moderate toxicity. Meyer et al¹⁶ in his study classified the toxicity of substance or chemical based on the concentration, i.e. $\leq 30 \text{ mg/L}$ = very toxic, $\text{LC}_{50} \leq 1000 \text{ mg/L}$ = moderate, $\text{LC}_{50} > 1000 \text{ mg/L}$ = not toxic. LC_{50} is calculated by determining the concentration of test compounds that can kill 50% population of shrimp larvae¹⁷.

CONCLUSION

The phytochemical screening of ethanolic extract of *Rhizophora mucronata* identified the presence of flavonoid, alkaloid, tannin, phenolic and quinone. The BSLT test showed that the ethanolic extract of *Rhizophora mucronata* has moderate toxicity.

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